

WHAT IS CLAIMED IS:

1 1. A method of qualifying a prostate cancer status in a subject
2 comprising:

3 (a) measuring at least one biomarker in a sample from the subject,
4 wherein the biomarker is selected from the group consisting of:

5 Marker EP1: 3448 ± 19 Da,

6 Marker EP2: 4036 ± 22 Da,

7 Marker EP3: 4361 ± 24 Da,

8 Marker EP4: 4413 ± 24 Da,

9 Marker EP5: 4639 ± 26 Da,

10 Marker EP6: 4749 ± 26 Da,

11 Marker EP7: 4827 ± 27 Da,

12 Marker EP8: 5666 ± 31 Da,

13 Marker EP9: 8445 ± 46 Da,

14 Marker EP10: 11744 ± 65 Da,

15 Marker EP11: 14696 ± 81 Da,

16 Marker EP12: 24184 ± 133 Da,

17 Marker EP13: 48308 ± 266 Da,

18 Marker EP14: 53830 ± 296 Da; and combinations thereof;

19 and,

20 (b) correlating the measurement with prostate cancer status.

1 2. The method of claim 1, wherein the prostate cancer status is
2 selected from the group consisting of prostate cancer (PCA), prostate intraepithelial
3 neoplasia (PIN), and benign prostate hyperplasia (BPH).

1 3. The method of claim 1, wherein the sample is prostate tissue
2 extract.

1 4. The method of claim 1, wherein measuring comprises determining
2 the mass of the protein.

1 5. The method of claim 4, wherein the mass of the protein is
2 determined by mass spectrometry.

1 6. The method of claim 1, wherein the sample is selected from the
2 group consisting of blood, serum, urine, prostatic fluid, seminal fluid, semen, and prostate
3 tissue.

1 7. The method of claim 5, wherein mass spectrometry is gas phase ion
2 spectrometry.

1 8. The method of claim 7, wherein gas phase ion spectrometry is laser
2 desorption ionization mass spectrometry.

1 9. The method of claim 1, wherein step a) further comprises detecting
2 the marker by immunoassay.

1 10. The method of claim 1, wherein
2 step a) further comprises:
3 i) fractionating the sample;
4 ii) binding a fraction of the sample to an adsorbent; and,
5 iii) comprises detecting the marker by gas phase ion spectrometry.

1 11. The method of claim 10, wherein the adsorbent is selected from the
2 group consisting of a hydrophilic adsorbent, a metal chelate adsorbent, and a strong anion
3 exchange adsorbent.

1 12. The method of claim 1, wherein
2 step a) comprises:
3 i) embedding a portion of a tissue specimen harvested from a
4 patient in OCT and freezing the specimen;
5 ii) obtaining cryosections from the tissue specimen;
6 iii) obtaining cell samples from the cryosections by laser capture
7 microdissection;
8 iv) mixing cell samples from step (d) with lysis buffer thereby
9 producing cell lysates;
10 v) diluting and vortexing the cell lysates,
11 vi) centrifuging the vortexed cell lysates thereby producing a
12 supernatant fraction;

13 vii) binding the supernatant fraction to an adsorbent; and,
14 viii) comprising detecting the marker using gas phase ion
15 spectrometry.

1 13. The method of claim 12, wherein the adsorbent of step a) is
2 selected from the group consisting of a hydrophilic adsorbent, a metal chelate adsorbent,
3 and a strong anion exchange adsorbent.

1 14. The method of claim 1, wherein the sample comprises the marker
2 EP8.

1 15. The method of claim 1, wherein the sample comprises the markers
2 EP2 and EP3.

1 16. The method of claim 1, wherein the sample comprises the markers
2 EP2 and EP5.

1 17. The method of claim 1, wherein the sample comprises the markers
2 EP3 and EP5.

1 18. The method of claim 1, wherein the sample comprises the markers
2 EP2 and EP6.

1 19. The method of claim 1, wherein the sample comprises the markers
2 EP3 and EP6.

1 20. The method of claim 1, wherein the sample comprises the markers
2 EP5 and EP6.

1 21. The method of claim 1, wherein the sample comprises the markers
2 EP2, EP3 and EP5.

1 22. The method of claim 1, wherein the sample comprises the markers
2 EP2, EP3 and EP6.

1 23. The method of claim 1, wherein the sample comprises the markers
2 EP2, EP3, EP4, EP5, EP6, and EP8.

1 24. The method of claim 8, wherein
2 step b) comprises:
3 i) generating data for each marker with the mass
4 spectrometer, the data comprising a mass/charge ratio and an amount determination for
5 each ion corresponding to each marker;
6 ii) transforming the data into computer-readable form; and,
7 iii) executing an algorithm with a programmable digital
8 computer,
9 wherein the algorithm determines closeness-of-fit between the computer-
10 readable data and a data set indicating a diagnosis of PCA, PIN, BPH or a negative
11 diagnosis.

1 25. A method for detecting at least one marker in a sample, the method
2 comprising:
3 a) obtaining a sample comprising at least one marker, where each
4 marker is selected from the group consisting of:
5 Marker EP1: 3448 ± 19 Da,
6 Marker EP2: 4036 ± 22 Da,
7 Marker EP3: 4361 ± 24 Da,
8 Marker EP4: 4413 ± 24 Da,
9 Marker EP5: 4639 ± 26 Da,
10 Marker EP6: 4749 ± 26 Da,
11 Marker EP7: 4827 ± 27 Da,
12 Marker EP8: 5666 ± 31 Da,
13 Marker EP9: 8445 ± 46 Da,
14 Marker EP10: 11744 ± 65 Da,
15 Marker EP11: 14696 ± 81 Da,
16 Marker EP12: 24184 ± 133 Da,
17 Marker EP13: 48308 ± 266 Da, and
18 Marker EP14: 53830 ± 296 Da; and,
19 b) detecting the marker by gas phase ion spectrometry.

1 26. The method of claim 25, wherein gas phase ion spectrometry is
2 laser desorption/ionization mass spectrometry.

1 27. The method of claim 22, wherein the sample comprises at least two
2 markers wherein each marker is differentially present in the sample.

1 28. The method of claim 26, further comprising:
2 c) generating data for each marker with the mass spectrometer, the
3 data comprising a mass/charge ratio and an amount determination for each ion
4 corresponding to each marker,
5 d) transforming the data into computer-readable form; and
6 e) executing an algorithm with a programmable digital computer
7 wherein the algorithm detects the amount determination in the computer-
8 readable data representing the marker and determines closeness-of-fit between the
9 computer-readable data and a data set indicating a diagnosis of PCA, PIN, BPH or a
10 negative diagnosis.

1 29. The method of claim 29, wherein the algorithm comprises an
2 artificial intelligence program.

1 30. The method of claim 29, wherein the artificial intelligence program
2 is a fuzzy logic, cluster analysis or neural network.

1 31. The method of claim 25, wherein step a) further comprises:
2 fractionating the sample by size exclusion chromatography or anion exchange
3 chromatography, and collecting a fraction that includes the marker or markers.

1 32. The method of claim 25, wherein step a) further comprises
2 contacting the sample with a substrate comprising an adsorbent that retains the marker
3 and removing unretained sample.

1 33. The method of claim 29, wherein the substrate is a mass
2 spectrometer probe comprising the adsorbent on a probe surface.

1 34. The method of claim 29, wherein the substrate is a resin, and step
2 a) further comprises placing the resin with the marker retained by the adsorbent on a mass
3 spectrometer probe.

1 35. The method of claim 29, wherein the adsorbent is selected from the
2 group consisting of a hydrophilic adsorbent, a strong anion exchange adsorbent and a
3 metal chelate adsorbent.

1 36. The method of claim 26, wherein
2 step a) further comprises:
3 i) providing a probe adapted for use with a mass
4 spectrometer comprising an adsorbent attached thereto;
5 ii) contacting the sample comprising the marker with the
6 adsorbent.

1 37. The method of claim 26, wherein
2 step a) further comprises:
3 i) providing a substrate comprising an adsorbent attached
4 thereto;
5 ii) contacting the sample comprising the protein with the
6 adsorbent;
7 iii) placing the substrate on a probe adapted for use with a
8 mass spectrometer.

1 38. The method of claim 36, wherein the adsorbent is a hydrophilic
2 adsorbent or a metal chelate adsorbent.

1 39. The method of claim 37, wherein the adsorbent is a hydrophilic
2 adsorbent comprising silicon oxide.

1 40. The method of claim 37, wherein the adsorbent is a metal chelate
2 adsorbent comprising copper.

1 41. The method of claim 37, wherein the adsorbent comprises an
2 antibody that specifically binds to the marker.

1 42. A purified marker selected from the group consisting of:
2 Marker EP1: 3448 ± 19 Da,
3 Marker EP2: 4036 ± 22 Da,
4 Marker EP3: 4361 ± 24 Da,

5 Marker EP4: 4413 ± 24 Da,
6 Marker EP5: 4639 ± 26 Da,
7 Marker EP6: 4749 ± 26 Da,
8 Marker EP7: 4827 ± 27 Da,
9 Marker EP8: 5666 ± 31 Da,
10 Marker EP9: 8445 ± 46 Da,
11 Marker EP10: 11744 ± 65 Da,
12 Marker EP11: 14696 ± 81 Da,
13 Marker EP12: 24184 ± 133 Da,
14 Marker EP13: 48308 ± 266 Da, and
15 Marker EP14: 53830 ± 296 Da.

1 43. The purified protein of claim 42, produced by a process
2 comprising:
3 i) fractionating a sample comprising the marker or markers by size
4 exclusion chromatography or anion exchange chromatography; and,
5 ii) collecting a fraction that includes the marker or markers.

1 44. The purified protein of claim 42, produced by a process
2 comprising:
3 i) microdissecting a cell sample comprising the marker by laser
4 capture microdissection, thereby producing isolated cells,
5 ii) lysing the isolated cells producing a cell lysate,
6 iii) centrifuging the cell lysate, thereby producing a cell supernatant
7 comprising the marker,
8 iv) contacting the cell supernatant to an adsorbent sufficient to
9 allow the adsorbent to bind the marker;
10 v) washing the adsorbent to remove unbound cell supernatant; and,
11 vi) eluting the marker from the adsorbent.

1 45. A kit comprising:
2 (1) an adsorbent attached to a substrate, wherein the adsorbent is
3 suitable for retaining a marker selected from the group consisting of:
4 Marker EP1: 3448 ± 19 Da,
5 Marker EP2: 4036 ± 22 Da,

6 Marker EP3: 4361 ± 24 Da,
7 Marker EP4: 4413 ± 24 Da,
8 Marker EP5: 4639 ± 26 Da,
9 Marker EP6: 4749 ± 26 Da,
10 Marker EP7: 4827 ± 27 Da,
11 Marker EP8: 5666 ± 31 Da,
12 Marker EP9: 8445 ± 46 Da,
13 Marker EP10: 11744 ± 65 Da,
14 Marker EP11: 14696 ± 81 Da,
15 Marker EP12: 24184 ± 133 Da,
16 Marker EP13: 48308 ± 266 Da, and
17 Marker EP14: 53830 ± 296 Da; and
18 (2) instructions for using the substrate to detect the marker.
19

1 46. The kit of claim 45, wherein the instructions include methods for
2 contacting a sample comprising the marker with the adsorbent.